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Comparing Prothrombin induced by vitamin K absence-II (PIVKA-II) with the oncofetal proteins Glypican-3, Alpha feto protein and Carcinoembryonic antigen in diagnosing hepatocellular carcinoma among Egyptian patients

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KEYWORDS

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Abstract *Background:* Hepatocellular carcinoma (HCC) is usually asymptomatic in the early stage and does not show elevated alpha-feto protein (AFP). AFP shows 60–80% sensitivity in diagnosing HCC.

Glypican3 (GPC-3) is an oncofetal protein that is only detected in HCC cells but not in benign liver tissues, while Carcinoembryonic antigen (CEA) is expressed in various neoplasms including HCC. Although, it is not specific for HCC.

Prothrombin induced by vitamin K absence-II (PIVKA-II) is an abnormal prothrombin protein that is increased in the serum of HCC patients. It has higher sensitivity and specificity compared to AFP. The aim of this study is to compare the clinical utility of PIVKA-II with GPC-3, AFP and CEA in diagnosing HCC.

Abbreviations: HCC, hepatocellular carcinoma; AFP, Alpha-feto protein; GPC-3, Glypican3; CEA, Carcinoembryonic antigen; PIVKA-II, Prothrombin induced by vitamin K absence-II

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Patients and methods: This study included 40 patients with HCC, 10 patients with cirrhosis as a benign control group, and 10 apparently healthy volunteers as normal controls.
Serum samples were subjected to routine laboratory investigations, measurement of CEA, AFP using MEIA technique (Axsym), glypican3, and PIVKA-II using ELISA technique in the sera of all patients and controls.
Results: All markers showed the highest results in the HCC group. Higher concentrations of PIVKA-II were detected in patients with splenomegaly, and in tumors with size (> 3 cm). Combination of Glypican-3 and PIVKA-II showed the highest sensitivity, while GPC-3 alone and combination of GPC-3 and AFP showed the highest specificity to differentiate HCC from liver cirrhosis and normal controls. GPC-3, PIVKAII, and combination of both showed the highest sensitivity, while GPC-3 alone showed the highest specificity to differentiate HCC from liver cirrhosis.
Conclusion: Glypican-3 is the only oncofetal antigen that showed comparable high diagnostic accuracy as PIVKA-II in diagnosing HCC among Egyptian patients.

Introduction

Hepatocellular carcinoma (HCC) is a major health problem [1]. It ranked 2nd most common cancer site among males and 7th among females in the National Cancer Institute (NCI), Cairo University, Egypt [2]. HCC is usually asymptomatic in the early stage and tends to be intravascularly and intrabiliary invasive. Moreover, early HCC does not show elevated alpha-feto protein (AFP) [3].

Oncofetal antigens are proteins produced during fetal life and disappear after birth. In cancer patients, these proteins reappear which demonstrates that certain genes are reactivated as the result of the malignant transformation of cells [4].

AFP is the only molecular marker widely used for the diagnosis of HCC. At a cutoff value of 20 ng/ml, serum AFP shows 60–80% sensitivity [5]. This sensitivity may decrease to about 40% for small tumors [6]. In addition, a significant increase in serum AFP level (20–200 ng/ml) is detected in a considerable number of patients with chronic liver disease [7].

Glypican3 (GPC-3) is an oncofetal protein member of the glypican family. It plays an important role in cell growth, differentiation and migration [8]. It is only detected in HCC cells but not in benign liver tissues [9]. Some studies investigated the role of GPC-3 as a marker for early stage of HCC [9–12]. They found it to be a sensitive and specific marker for the diagnosis of early HCC.

Carcinoembryonic antigen (CEA) is expressed in various neoplasms of endodermal origin including HCC. However, serum CEA levels alone are not specific for HCC [13].

Prothrombin induced by vitamin K absence-II (PIVKA-II) is an abnormal prothrombin protein that is increased in the serum of HCC patients as a result of an acquired defect in the posttranslational carboxylation of the prothrombin precursor in malignant cells [4]. Many studies showed that PIVKA-II has higher sensitivity and specificity compared to AFP in differentiating HCC from other chronic liver diseases [14–17].

The aim of this study is to compare the clinical utility of PIVKA-II with the oncofetal antigens; GPC-3, AFP and CEA in differentiating HCC patients from benign cirrhotic patients and normal controls, also to compare such markers with different prognostic factors of HCC.

Patients and methods

Patients

This study included 40 newly diagnosed HCC patients, all cases who were presented to the outpatients' clinic at the NCI, Cairo University, as well as the National Liver Institute, Cairo over a period of consecutive 9 months from January to September 2012, and were eligible for the study were included. Their age ranged from 44 to 77 years with a median of 59. They were proven to be HCC by computed tomography (CT) or magnetic resonance imaging (MRI).

Exclusion criteria: Prolonged obstructive jaundice, intrahepatic cholestasis with vitamin K deficiency and intake of warfarin or antibiotics.

The study also included 10 patients with cirrhosis as a benign control group who were diagnosed on the basis of clinical and radiological evidence. They were 8 males and 2 females. Their age ranged from 44 to 72 years with a median of 57. Also, 10 apparently healthy volunteers were included as normal controls; they were 5 males and 5 females, their age ranged from 36 to 44 years with a median of 40.

A written consent from all patients according to the international ethics committee guidelines, and IRB approval were obtained.

Blood samples from patients and controls were subjected to the following:

(1) Liver function tests using Beckman CX9 auto-analyser. Prothrombin time and concentration using Siemens turbitimer [18].
(2) Tumor Markers: AFP [19], CEA [20] were done using Axsym based on the microparticle enzyme immunoassay (MEIA) technology.
(3) PIVKA-II was done using BlueGene Biotech, Shanghai, China by ELISA technique.
(4) Glypican-3 was done using Uscn Life Science Inc. Wuhan, China by ELISA technique.

Haemolysed and lipemic samples were excluded.

Statistical analysis

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). For quantitative data,

comparison between two groups was done using Mann–Whitney test (non-parametric *t*-test). Comparison between 3 groups was done using Kruskal–Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" on rank of variables was used for pair-wise comparison. Spearman-rho method was used to test the correlation between numerical variables. The Receiver Operating Characteristic (ROC) curve was used for prediction of cutoff values. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated for the different markers used. *P*-value <0.05 was considered significant.

Table 1 Patients' characteristics of the hepatocellular carcinoma and cirrhosis groups.

Characteristic	*N* (40)	Percentage
Sex		
Males	32	80
Females	8	20
Child's grade		
Grade A	9	22.5
Grade B	22	55
Grade C	9	22.5
Stage		
Stage I	2	5
Stage II	30	75
Stage III	8	20
Hepatomegaly	23	57.5
Splenomegaly	11	27.5
Ascites	20	50
Edema	3	7.5
PVT	8	20
Number of masses		
1 mass	19	47.5
2 masses	9	22.5
3 masses	10	25
4 masses	2	5
Hepatitis markers		
Hepatitis B	7	17.5
Hepatitis C	26	65
Non B non C	7	17.5
Size of mass		
1–3 cm	17	42.5
More than 3	23	57.5
Cirrhosis cases		
Sex		
Males	8	80
Females	2	20
Hepatomegaly	6	60
Splenomegaly	2	20
Hepatitis markers	Null	

Results

Patients' characteristics of the HCC and cirrhosis groups are mentioned in Table 1.

Glypican-3, AFP, and PIVKA-II showed the highest results in the HCC group followed by the cirrhotic and then the normal control group ($P < 0.001$) each. Also CEA showed the same results ($P = 0.024$) (Table 2).

On comparing the studied markers with some of the prognostic factors of HCC (age, sex, hepatomegaly, splenomegaly, ascites, portal vein thrombosis, number of masses in the liver, tumor size, grade, and stage), significantly higher concentrations of PIVKA-II levels were detected in patients with splenomegaly, and large tumor size (>3 cm) ($P = 0.018$, $P < 0.001$), respectively (Table 3).

Comparison of the studied markers according to HCV and HBV positivity revealed non-significant results.

On differentiating HCC from cirrhosis and normal controls, GPC-3, PIVKA-II, AFP, and combination of GPC-3 and PIVKA-II at cut-off levels of 4.9 ng/ml, 1.2 ng/ml, 40.5 ng/ml, and 4.9 ng/ml & 1.2 ng/ml showed sensitivities of 95%, 97.5%, 82.5% and 100% and specificities of 95%, 90%, 85% and 90%, respectively (Table 4).

To differentiate between HCC and liver cirrhosis, best chosen cutoff values were 4.9 ng/ml, 1 ng/ml, and 4.8 ng/ml and 1 ng/ml for GPC-3, PIVKA-II, and combination of GPC-3 and PIVKA-II, respectively. Sensitivities were 100% each, while specificities were 90%, 80%, and 60%, respectively (Table 5).

Discussion

The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years [21]. Being a disease with fast infiltrating growth makes it urgent to find sensitive markers for early diagnosis and monitoring of recurrence [22].

In this study, the chosen cutoff values to differentiate HCC from normal controls and cirrhosis patients for Glypican-3, PIVKA II, AFP, CEA, and combinations of Glypican-3 and PIVKA-II were 4.9 ng/ml, 1.2 ng/ml, 40.5 ng/ml, 1.7 ng/ml, 4.9 ng/ml & 1.2 ng/ml, respectively. At these chosen cutoff values, high concentration of GPC-3, PIVKA-II, AFP and CEA was detected in 95%, 98%, 82.5%, and 85% of HCC patients, and 10%, 20%, 30%, and 70% of cirrhosis patients, respectively. As regards normal controls, GPC-3, PIVKA-II,

Table 2 Comparison of the studied tumor markers in different groups.

	HCC group	Liver cirrhotic group	Normal controls group	*P*-value
PIVKA II (ng/ml)	4.2 (1–15.7) a,b	0.8 (0.13–3.31) a	0.44 (0.07–1.07) b	**<0.001***
Glypican-3 (ng/ml)	7.7 (4.9–11) c,d	2.74 (1.99–5.93) c	0.99 (0.86–1.67) d	**<0.001***
Alpha feto protein (ng/ml)	146.5 (1.9-500000) e,f	15 (2.5–77) e	3.4 (1.6–25) f	**<0.001***
Carcinoembryonic antigen (μg/l)	3.1 (2.2–4.1)g	3.2 (1.7–3.7)	1.8 (1–2) g	**0.024***

Groups median sharing the same letter show statistically significant comparisons.
HCC, Hepatocellular carcinoma; PIVKAII, Prothrombin induced by vitamin K absence.
Median and interquartile ranges in parenthesis.
* Significant.

Table 3 Comparison of Glypican-3, Prothrombin induced by vitamin K absence (PIVKAII), Alpha feto protein and Carcinoembryonic antigen with different prognostic factors in the hepatocellular carcinoma group.

	Glypican-3 (ng/ml)	*P*-value	PIVKA-II (ng/ml)	*P*-value	AFP (ng/ml)	*P*-value	CEA (μg/l)	*P*-value
Splenomegaly								
Absent (n = 29)	8.04 (5–11)		3.61 (1–11)		183 (65–421)		2.9 (1.9–4.1)	
Present (n = 11)	7.32 (5–11)		5.97 (2–16)		97 (43–940)		3.1 (2.7–4.1)	
		0.929		**0.018***		0.832		0.363
Tumor size								
Up to 3 cm (n = 17)	7.01 (5–11)		3.10 (1–5)		201 (59–505)		2.7 (1.6–3.7)	
More than 3 cm (n = 23)	9.01 (5–11)		5.97 (2–16)		121 (35–346)		3.3 (2.5–4.1)	
		0.075		**< 0.001***		0.557		0.827

PIVKAII, Prothrombin induced by vitamin K absence; AFP, Alpha feto protein; CEA, Carcinoembryonic antigen; PV thrombosis, Portal vein thrombosis.
Median and interquartile ranges in parenthesis.
* Significant.

Table 4 Diagnostic accuracy of the different studied tumor markers to differentiate between malignant cases and benign and normal controls.

	GPC-3 (cutoff 4.9 ng/ml)	PIVKA-II (cutoff1.2 ng/ml)	AFP (cutoff 40.5 ng/ml	CEA (cutoff 1.7 μg/l)	Combined GPC-3 and PIVKA II (cutoff 4.9 ng/ml & 1.2 ng/ml)	Combined GPC-3 and AFP (cutoff 4.9 ng/ml & 40.5 ng/ml)	Combined sPIVKA-II & AFP (cutoff 1.2 ng/ml & 40.5 ng/ml)
Sen %	95	97.5	82.5	85	100	80	75
(95% CI)	(86–99)	(89–100)	(70–91)	(70–94)	(93–100)	(64–90)	(58–87)
Spe %	95	90	85	20	90	95	90
(95% CI)	(85–99)	(79–96)	(73–93)	(2–55)	(79–96)	(75–99)	(68–98)
PPV %	97	95	91.7	81	95	97	94
(95% CI)	(89–100)	(85–99)	(81–97)	(65–91)	(86–99)	(84–99)	(79–99)
NPV %	90.5	95	71	25	100	70	64
(95% CI)	(79–96)	(85–99)	(56–82)	(3–65)	(93–100)	(49–86)	(44–81)
DA %	95	95	83	72	97	85	80
(95% CI)	(88–98)	(88–98)	(74–89)	(62–80)	(91–99)	(76–91)	(71–87)

Sen, Sensitivity; Spe, Specificity; PPV, Positive predictive value; NPV, Negative predictive value; DA, Diagnostic accuracy; 95%; CI, 95% confidence interval; GPC-3, glypican-3; PIVKAII, Prothrombin induced by vitamin K absence; AFP, Alpha feto protein; CEA, Carcinoembryonic antigen.

Table 5 Diagnostic accuracy of the studied markers to differentiate between hepatocellular carcinoma and liver cirrhosis cases.

	Glypican 3 (cutoff 4.8 ng/ml)	Glypican 3 (cutoff 4.9 ng/ml)	PIVKA-II (cutoff 1 ng/ml)	Combined GPC-3 & PIVKA II (cutoff 4.8 ng/ml & 1 ng/ml)	AFP (cutoff 20 ng/ml)
Sen%	100	100	100	100	90
(95% CI)	(89–100)	(89–100)	(91–100)	(91–100)	(76–97)
Spe%	80	90	80	60	60
(95% CI)	(44–96)	(54–99)	(44–97)	(26–87)	(26–87)
PPV%	95	97.5	95	91	90
(95% CI)	(83–99)	(86–100)	(83 –99)	(78–97)	(76–97)
NPV%	100	100	100	100	60
(95% CI)	(60–100)	(63–100)	(63–100)	(54–100)	(26–87)
DA%	96	98	84	80	80
(95% CI)	(90–98)	(93–99)	(75–90)	(71–87)	(71–87)

Sen, Sensitivity; Spe, Specificity; PPV, Positive predictive value; NPV, Negative predictive value; DA, Diagnostic accuracy 95%; CI, 95% confidence interval; PIVKAII, Prothrombin induced by vitamin K absence; AFP, Alpha feto protein.

and AFP levels were below the chosen cutoff values, while CEA level was above the chosen cutoff value in 50% of normal controls.

Only 81% of GPC3 positive cases and 82% of PIVKA-II positive cases showed elevated AFP levels. All HCC cases positive for GPC3 were positive for PIVKA-II, which shows

that both markers are almost equally highly sensitive and specific for the diagnosis of early HCC compared to AFP and CEA.

Nakatsura et al. [23] stated that GPC-3 could be detected in 40–53% of HCC patients and 33% of AFP sero-negative HCC patients, while Liu and coworkers [9] found that serum GPC3 level was higher than 300 ng/l in 50% of early HCC patients, although their serum AFP level was below 100 μg/l. Shafizadeh et al. [24] found GPC3 positive cells in 90% of patients with their serum AFP level < 400 μg/l.

Oncofetal antigens are proteins produced during fetal life, disappear after birth, and reappear in cancer patients [4].

The serum levels of all markers in this study were found to be significantly higher in the HCC followed by the cirrhosis then the normal control groups which is in accordance with Nakatsura et al. (2003) [23] who reported high concentrations of GPC-3, PIVKA-II and AFP in the HCC, followed by the cirrhotic and then the normal control group.

Another study done by Zachary et al. [16] revealed a significant elevation in both PIVKA-II and AFP in the HCC group compared to the benign and normal control groups. Hippo et al. [25] demonstrated detectable low levels of GPC-3 in the sera of normal controls as we did, this may be attributed to the fact that, GPC-3 can only be detected in adults in a limited number of tissues, including lung, ovaries, mammary epithelium, and mesothelium [26]. Depending on the tissue, Glypican-3 displays a very different pattern of expression during tumor progression. In cancers originated from tissues that are CPC-3 positive in adults, the expression of GPC-3 is reduced during tumor development. On the other hand, in tumors originated from tissues that only express GPC-3 in the embryo, GPC-3 expression tends to reappear on malignant transformation [11].

Comparing the studied markers with some of the prognostic factors of HCC revealed significant results between elevated serum levels of PIVKA-II with splenomegaly ($P = 0.018$), and tumor size (> 3 cm) ($P < 0.001$).

Similar results were obtained by Zachary et al. [16]. Also, Sharma et al. [15] found that AST and tumor size were two factors that independently affected PIVKA-II levels in HCC patients.

PIVKA-II also has been reported to predict the progression of HCC as higher PIVKA-II levels were accompanied by higher frequency of intrahepatic metastasis, portal or hepatic vein tumor thrombosis and capsular infiltration [16]. In our study, however, we did not find any relationship between the portal vein invasion and PIVKA-II levels.

We could not detect any significant relation between GPC-3 or AFP with tumor size, which shows that PIVKA-II is more indicative about the tumor bulk, hence can be more suitable than AFP for earlier diagnosis of HCC. Similarly, Ozkan et al. [27] found no correlation between GPC-3 levels and prognostic parameters in patients with HCC. Contrarily, a positive correlation was found between serum levels of AFP and GPC-3 with both tumor size and portal vein invasion by El-Shenawy et al. [28].

GPC-3, PIVKA-II, AFP, and CEA showed no significant results with the different stages of HCC. Zachary et al. [16] reported similar results with regard to AFP, while PIVKA-II showed significant results. Contrary to our results, Youssef et al. [10] reported significant results between GPC-3 and the staging of HCC.

It has been documented that Egypt has one of the highest prevalence of HCV infection in the world [29]. So, we compared the serum levels of the studied markers with the positivity of HBV and HCV infection which revealed no significant correlations between GPC3 and PIVKA-II with HCV, or HBV infections, or with the markers of hepatic injury as AST, ALT, albumin, and prothrombin time. This is a good indicator of the high specificity of GPC-3 and PIVKA-II in our Egyptian HCC versus non HCC hepatitis and hepatic injury patients, as in some patients having chronic hepatitis, and liver cirrhosis, AFP level can reach 2500 μg/l in around 20–25% [30].

Our results are in agreement with Capurro et al. [31], and Nakastura et al. [23] who reported that GPC-3 was present in the serum of HCC patients, but was undetectable in all patients with hepatitis and healthy individuals. No significant changes were observed concerning the levels of PIVKA-II in HCV in a study by Zachary et al. [16].

On differentiating HCC from cirrhosis and normal controls, GPC-3, PIVKA-II, and AFP at cut-off levels of 4.9 ng/ml, 1.2 ng/ml, and 40.5 ng/ml showed sensitivities of 95%, 97.5%, and 82.5% and specificities of 95%, 90%, and 85%, respectively.

Gomaa et al. [12], El-Shenawy et al. [28], and Youssef et al. [10] reported a wide range of sensitivities for GPC3 (90.3%, 63.5% and 82.5%), and specificities (98%, 70%, and 95%) at cutoff values of 5.41 ng/ml, 19 ng/ml, and 4.6 ng/ml, respectively. As for AFP, sensitivities were 77.4%, 76.5%, and 80%, specificities were 60%, 82%, 90% at cutoffs (42.32 ng/ml, 78 ng/ml, and 66 ng/ml), respectively in their studies to differentiate HCC from liver cirrhosis and normal controls. Also Suriawinata et al. [11] reported 100% specificity for GPC3 in HCC patients which disappeared from the sera of three patients after surgical treatment.

As for PIVKA-II, different cut-off values (40 mAU/ml, 63 mAU/ml, and 42.74 ng/ml) have been proposed by different authors in different ethnic populations [32,4,33]. Such different results might be related to the etiologic difference underlying liver disease and the ethnicity of the population studied [15].

Regarding the diagnostic performance of PIVKA-II, studies by Zachary et al. [16], Sharma et al. [15], Choi et al. [17], and Singhal et al. [34] showed sensitivities of 100%, 80%, 60%, and 89%) and specificities of 100%, 92%, 95%, and 86.7%, at cut-off values of 39.6 ng/ml, 9.2 ng/ml, 4 ng/ml, and 12.5 ng/ml, respectively. As for AFP, they showed sensitivities of 73.3%, 72.9 ng/ml, and 78.9% and specificities of 75%, 65.8%, and 84.6% at cutoff values of 22.3 ng/ml, 13.02 ng/ml, and 10 ng/ml, respectively. They concluded that PIVKA-II proved to be superior to AFP in early detection of HCC.

As for AFP, Cheng et al. [35], Jackubovic and Jothy [36] and Zhou et al. [37] stated that the cut-off value of AFP is fluctuant in different ethnic groups due to the diverse living circumstances, the diversity of patient populations examined, varying study designs and differing cut-off values for normality. They also reported that AFP is more useful in detecting HCC patients with non-viral etiology. Thus, serum AFP level plays a limited role in early diagnosis of HCC which is consistent with our results.

Given the recognized heterogeneity of HCC, it is unlikely that a biochemical marker that is specifically expressed in 100% of HCCs will be identified. However, it is possible that a combination of 2 or 3 markers will increase the sensitivity of detection [31].

Several studies have shown that Glypican-3 and PIVKA-II are superior to AFP in early detection of HCC, being highly sensitive and specific [16,11]. So, we tested both markers in combination which improved the sensitivity and the specificity to 100% and 90%, respectively.

Other studies performed combination of Glypican-3 and AFP. Sensitivities were between 84–92% and specificities between 90–95% [12,38,10].

A combination of PIVKA-II and AFP resulted in an improvement in specificity, but the sensitivity decreased in a study by Sharma et al. [15].

Although, most liver nodular lesions are benign, they may mimic malignant liver lesions [39]. Therefore, the differential diagnosis between HCC and benign mimickers is difficult [40].

To differentiate between HCC and liver cirrhosis, we chose 4.8 ng/ml as a cutoff level for glypican3; it showed 100% sensitivity and 80% specificity, while elevating the cutoff by 0.1 ng/ml raised the specificity to 90% while the sensitivity remained 100%. Regarding PIVKA-II, a cutoff 1 ng/ml gave sensitivity and specificity of 100% and 80%, respectively. Combining glypican3 at a cutoff 4.8 ng/ml and PIVKA-II at a cutoff 1 ng/ml gave a high sensitivity of 100%, but lowered the specificity to 60%.

Some authors also chose different cutoff levels to differentiate between HCC and cirrhosis. El-Shenawy et al. [28], chose >19 ng/ml as the best cutoff for sGPC-3 which yielded 63.5% sensitivity, and 70% specificity.

Regarding the cirrhotic patients in the current study, one of them showed elevated serum levels of GPC3, PIVKA-II and AFP level was 67 ng/ml. This patient was diagnosed as HCC 9 months later, which indicates that GPC-3 and PIVKA-II can be considered as sensitive markers for follow up of cirrhotic patients, to detect early development of HCC.

Consistently, Hippo et al. [25] demonstrated that during the follow-up of their cirrhotic patients having detectable GPC-3 levels, HCC developed within 6 months among considerable number of patients with neither significant change of serum AFP levels nor in abdominal ultrasonography.

In this study, no significant correlations were detected among the four markers whether in the HCC or the cirrhotic group, which is in agreement with other authors [23,31,25] who reported the lack of correlation between GPC-3 and AFP in HCC patients. They have also found that the simultaneous use of both markers significantly increased the sensitivity for HCC diagnosis.

Conclusion

GPC-3 is the only oncofetal antigen that showed comparable diagnostic performance to PIVKA-II. Both markers individually and in combination are promising diagnostic markers for HCC and for follow up of cirrhotic patients among Egyptian patients. Although CEA, AFP, and GPC-3 belong to the group of oncofetal antigens, they did not show any significant correlation between each other or PIVKA-II which improves the sensitivity of HCC detection.

Conflict of interest

None declared.

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